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DELAYED NAUPLIAR DEVELOPMENT OF MEIOBENTHIC COPEPODS[1]

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As part of our continuing studies on the meiofauna of the North Inlet Estuary, Georgetown, South Carolina, U. S. A., we have been culturing several harpacticoid copepods in the laboratory. In the course of routine culture examination and flow chart analyses of previous cultures, we observed the continual appearance of stage 1–3 nauplii in a given culture for periods up to seven weeks after an isolated female hatched her eggs. This observation then spurred us to establish additional, rigorously controlled cultures specifically designed to observe the frequency of naupliar appearance, and indeed, in these controlled cultures as well, naupliar development was delayed. In all cases the cultures were set up with particular care to insure that no extraneous nauplii were introduced with the gravid female. As soon as the female became gravid again or her offspring matured to adulthood, they were removed, thus eliminating the possibility of that female or a F_1 (or later) producing the observed nauplii.

What is so striking is the delayed development of the nauplii. An original gravid female hatches all her eggs within a 48-hour period (the egg sac disappears after the hatch); thus, the continual appearance of nauplii must be due to their differential growth. If this is the case, it would prove to be a distinct ecological advantage to increase the ability of the species to extend its occupation of an area. We have encountered what appears to be the same "time-released" phenomenon in four species. Herewith we report on this phenomenon in these four species and speculate on its adaptive significance.

Materials and Methods

Copepods were collected regularly during 1972–75 from subtidal sands and muds in the North Inlet Estuary, Georgetown, S. C. (30° 20′ N, 79° 10″ W) by scooping the upper 2–5 cm of sediment into plastic buckets. The buckets were filled to one-half their volume, capped and transported to the laboratory in air conditioned vehicles at a constant temperature. Upon returning to the laboratory those species which had a majority of ovigerous females (indicating ongoing reproduction in field) were individually sorted from the sediment using a mouth pipette and placed in culture dishes (8.3 cm diameter). In the original ("non-controlled") cultures ten mg of untreated natural sediment from the sampling site (from which all organisms except bacteria and algae had been manually removed) were placed in the culture dish and covered (5 cm) with 30 ‰ Millipore-filtered sea water. Individual ovigerous copepods were then introduced into this natural substrate. In the second

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("controlled") cultures, the ovigerous copepods were placed in sediment that had been frozen prior to use to remove residual life and the sediment was overlain with 5 cm of 30 ‰ Millipore-filtered sea water which had been heated to 70° C and conditioned at 18° C prior to use. All cultures were then placed in controlled environmental chambers with temperature and light/dark cycles the same as in the field. Salinity was maintained at 30 ‰ by the addition of distilled water to compensate for evaporation and monitored using a hand-held refractometer. Food, such as cultured benthic pennate diatoms (*Navicula directa*, *N. pelliculosa*) or natural ciliate-bacterial population, was introduced with ovigerous females; the cultures were never allowed to become food-limited.

In both the "controlled" and "non-controlled" cultures one ovigerous copepod was placed in each dish and observed daily until all her eggs had hatched. In the "non-controlled" cultures the female was allowed to remain in the culture until she again produced eggs. Since female harpacticoids are iteroparous [*i.e.*, stored sperm from one insemination is capable of fertilizing up to eight broods (Johnson & Olsen, 1948], the fact that an individual female became gravid again was expected. Upon noting internal egg development (prior to formation of the external egg sac), the female was removed from the culture; or if the female was not observed daily (*e.g.*, over a weekend) and the egg sac formed, she was in all cases removed to a new culture prior to complete development of the eggs. Furthermore, when the offspring reached the 4th–6th copepodite stage, they were removed to separate culture dishes. In the "controlled" cultures the female was removed immediately after hatching her first brood. Therefore, only the mother was present as an adult in the "non-controlled" cultures and, except during the time of hatching, an adult was never present in the "controlled" cultures.

After the female had completed hatching and was removed, the "controlled" cultures were examined daily and all nauplii enumerated as soon as they exhibited movement. "Non-controlled" cultures were examined at regular intervals not exceeding three days. The notation N_1–N_3 is used to note the appearance of nauplii because we could not distinguish between N_1, N_2, or N_3 stages in the culture dish. The only differences between N_1 and N_3 are a small size differential (about 20 μ) and the number of anal spines (see Rosenfield and Coull, 1974, for morphology of copepod developmental stages). However, we were able to distinguish N_1–N_3 from N_4–N_6 by observing naupliar movement. N_1–N_3 nauplii simply "wiggle" from grain to grain of sediment on the bottom of the culture dish, whereas N_4–N_6 nauplii actively crawl across the dish and rapidly swim up and down in the overlying water. Such differences in movement are obvious and thus make separation of the two naupliar classes a relatively simple procedure.

Results

Table I and Figure 1 summarize the delayed naupliar developmental data for the "non-controlled" cultures, and Table II and Figure 2 for the "controlled" cultures for the four species: *Paronychocamptus* sp. (see Coull, 1976, for description), *Microarthridion littorale* (Poppe), *Thompsonula hyaenae* (I. C. Thompson), and *Pseudobradya pulchella* Sars. One must realize that our original cultures ("non-controlled") were designed to rear copepods from egg to adult—not to examine the timing of naupliar growth. It was not until we completed a thorough analysis of

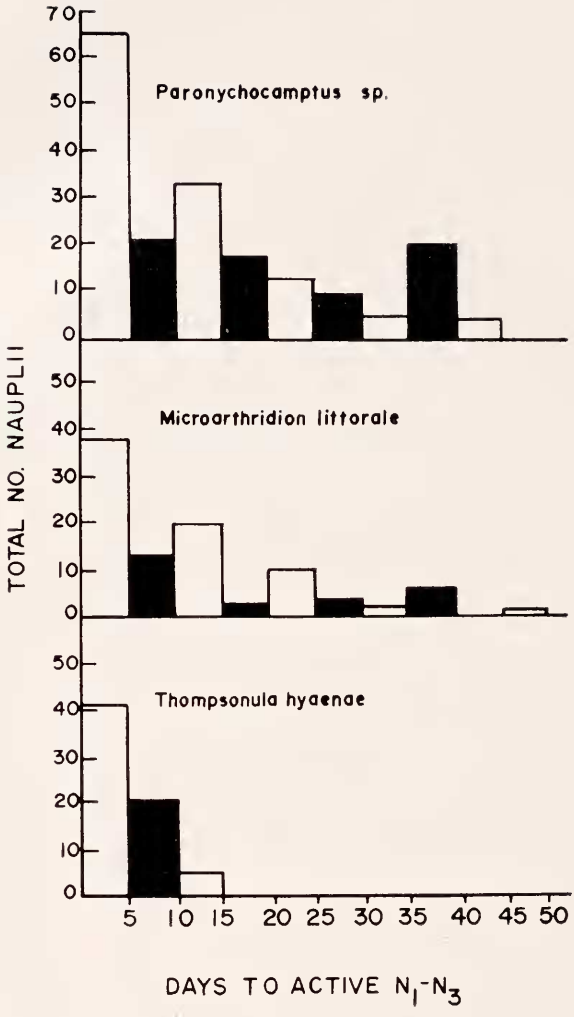

FIGURE 1. Summary data of the appearance of active stage 1–3 nauplii with time in three species of harpacticoid copepods in "non-controlled" cultures.

each individual female's offspring that we encountered the delayed naupliar development phenomenon. At this time we decided to more carefully culture the animals and examine the timing of naupliar activity. Thus the "controlled" cultures series was set up using species available in the field in adequate numbers during that season, *i.e.*, *Paronychocamptus* and *Pseudobradya*.

The data in Tables I and II are presented in a much-reduced form. We have developed flow charts of larval development for each individual but to present them would unduly clutter our report with charts not easily decipherable. One example should serve to illustrate the point. An ovigerous *Paronychocamptus* sp. (#288—

TABLE I

Summary data on naupliar development of three cultured ("non-controlled") harpacticoid copepods. Column 3 is particularly relevant in that it gives the days on which N_1–N_3 appeared in the culture and the number of N_1–N_3 on each day. Day 1 is the day of the hatch. Note that N_1–N_3 nauplii continued to appear up to 50 days (Microarthridion littorale, *#32) after the hatch.*

Species and culture number	Number of eggs	Days to N_1–N_3 appearance (and number of N_1–N_2 each day)	% mortality prior to N_1–N_3 $1 - \frac{\text{Number } N_1\text{–}N_3}{\text{Number eggs}} \times 100$
Paronychocamptus sp.			
288	20	5 (5), 8 (3), 14 (7), 28 (3), 41 (1)	5
288a	17	5 (5), 12 (3)	53
288b	17	16 (2), 36 (5)	59
288i	18	5 (1), 10 (8)	50
310a	18	5 (4)	78
310b	19	4 (5), 12 (1)	68
310c	18	6 (5), 18 (1)	67
326	20	13 (7)	65
330	18	15 (1), 31 (4), 44 (1)	67
336a	21	5 (7), 13 (5), 26 (4), 40 (4)	5
336b	20	5 (5), 36 (7)	40
339	19	4 (5)	74
347	18	2 (3), 5 (2), 21 (2), 28 (1), 42 (2)	48
348	19	16 (2), 18 (2), 20 (4)	58
349	18	2 (3), 5 (5), 18 (3), 25 (3), 36 (3)	5
347a	18	5 (4), 11 (4), 15 (2), 24 (2), 28 (1), 38 (1)	22
406	20	2 (2), 12 (5), 14 (10), 21 (1)	10
407	18	4 (2), 18 (4), 24 (2)	56
408	18	2 (8), 5 (3), 8 (2), 18 (2), 20 (1)	0
409	19	3 (6), 6 (2), 21 (1)	53
$\bar{x} \pm$ S.D.	18.6 ± 1.1		43.7
Microarthridion littorale			
32	20	2 (5), 11 (2), 25 (2), 40 (6), 50 (1)	20
32a	20	2 (3), 8 (3), 13 (1)	65
351	20	2 (4), 5 (2), 8 (3), 12 (3)	40
352	18	5 (6), 8(4), 12 (2), 20 (2)	22
353	18	2 (4), 8 (3), 12 (3), 22 (2)	33
354	18	2 (6), 12 (3), 23 (1), 31 (2)	33
355	20	5 (4), 12 (3), 22 (3), 30 (1)	45
356	20	5 (4), 12 (3), 22 (2), 30 (2)	45
$\bar{x} \pm$ S.D.	19.3 ± 1.0		37.9 ± 14.4
Thompsonula hyaenae			
27	25	4 (9), 8 (6), 10 (3), 13 (2)	20
27a	27	1 (7), 4 (12), 7 (3), 13 (3)	7
69	27	2 (13), 6 (8)	22
$\bar{x} \pm$ S.D.	26.3 ± 1.1		16.3

TABLE II

Summary data of naupliar development of two cultured ("controlled") harpacticoid copepods. Column 3 is particularly relevant in that it gives the days on which the N_1–N_3 nauplii appear in the culture and the number of N_1–N_3 each day. Day 1 is the day of the hatch.

Species and culture number	Number of eggs	Days to N_1–N_3 appearance (and number of N_1–N_3 each day)	% mortality prior to N_1–N_3 $1 - \frac{\text{Number } N_1\text{–}N_3}{\text{Number eggs}} \times 100$
Paronychocamptus sp.			
417	17	1 (3), 6 (5)	53
419	17	4 (2), 14 (1)	82
430	18	8 (1), 15 (2), 24 (2)	71
446	19	3 (14), 12 (2)	16
447	19	4 (4), 6 (6), 12 (1), 20 (1)	37
447a	18	8 (3), 12 (2), 16 (1)	67
448	19	3 (4), 12 (2)	47
449	19	4 (7), 6 (6)	32
450a	17	2 (5), 7 (1), 20 (1)	59
454	19	6 (9), 24 (1), 34 (2)	29
455	19	4 (8), 12 (4), 14 (2), 22 (2)	16
456	19	6 (8), 12 (2)	47
457	19	4 (8), 12 (4), 24 (5)	11
458	19	4 (1), 12 (2), 20 (2), 30 (1)	68
459	19	4 (8), 12 (5)	32
460	19	1 (3), 4 (8), 12 (4), 20 (2)	11
462	20	2 (2), 6 (8), 12 (2), 22 (2)	30
473	19	5 (2), 12 (4)	68
$\bar{x} \pm$ S.D.	18.6 ± 0.8		43.1
Pseudobradya pulchella			
463	15	4 (1), 10 (2), 20 (2)	67
470	16	4 (6), 15 (2), 25 (3), 30 (1)	38
472	15	8 (3), 12 (4) 20 (1)	47
475	16	3 (5), 6 (1)	63
476	15	3 (6), 6 (3), 15 (1)	33
477	15	5 (2), 15 (4), 22 (1)	54
478	15	3 (4), 6 (2), 15 (4), 26 (1)	27
479	16	3 (6), 5 (4), 15 (2), 24 (3)	7
480	15	3 (3), 5 (4), 15 (3)	33
$\bar{x} \pm$ S.D.	15.3 ± 0.5		41.0

from Table I) was isolated on 10/16/73; she hatched her 20 eggs on 10/18/73; five stage 2–3 nauplii appeared on 10/23/73; and two reached adulthood on 11/6 and 11/8/73, respectively—the others died. The two adults were then removed. On 10/26, we observed three more stage 1–3 nauplii, and one of these reached adulthood on 11/17/73. On 11/1 (14 days after the hatch in the same original culture) seven other stage 1–3 nauplii were found, three of which reached adulthood between 12/3/73 and 12/6/73. Again all were removed before copulation. On 11/15 (28 days after the hatch), three more stage 1–3 nauplii appeared, repeating the same procedure as before, and on day 41 (11/28/73) we encountered another stage 1–3 nauplii, which subsequently died. Further naupliar development was not observed after 11/28 in the original culture. *Paronychocamptus* normally carries

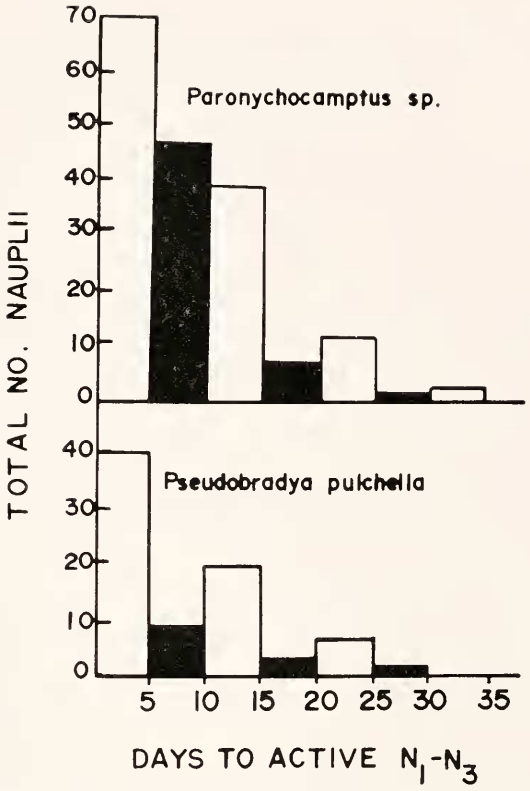

FIGURE 2. Summary data of the appearance of active stage 1–3 nauplii with time in two species of harpacticoid copepods in "controlled" cultures.

17–21 eggs (this particular individual had 20) and 19 nauplii which appeared at stage 1–3 during the 6-week period were accounted for.

The histograms, Figures 1 and 2, also illustrate that N_1–N_3 nauplii continued to appear in the cultures up to 50 days after the hatch. The greatest number of N_1–N_3 nauplii in all four species occurred in the first 1–5 days with an irregular decline in numbers subsequently. The histograms for three of the four species (*Thompsonula*, Figure 1, excepted) show secondary N_1–N_3 peaks at irregular intervals. Whether these secondary peaks are real or merely a function of small sample size is unknown. Nevertheless, the point is still made that there is obviously a prolongation of the N_1–N_3 stages in these four benthic harpacticoids. We have been relatively successful in culturing *Paronychocamptus* sp., which accounts for more data on this species. Naupliar growth is 38 (20 "non-controlled", 18 "controlled") cultures illustrates that the delayed naupliar development is a regular occurrence in this species. In fact, in every *Paronychocamptus* culture that was successfully maintained, delayed development occurred. We do not have data on as many single cultures of the other three species for two reasons: 1) successful rearing of *M. littorale*, and *T. hyaenae* was erratic, and 2) many of the earlier cultures

of *Microarthridion, Thompsonula* and *Pseudobradya* were not checked frequently enough to allow for careful naupliar enumeration. Even so, delayed naupliar development is also apparent in these three species (Tables I and II, and Figures 1 and 2).

Discussion

In the literature dealing with growth, culturing and development of harpacticoid copepods (*e.g.*, Johnson and Olsen, 1948; Huizinga, 1971; Betouhim-el and Kahan, 1972; Volkmann-Rocco, 1972; Rosenfield and Coull, 1974; Smol and Heip, 1974), there is only one hint of differential naupliar growth. Smol and Heip (1974) suggest delayed naupliar stages and contend that the delay is in response to increased temperature. They do not, however, give any data on the length of the individual stages and only state, "In the case of *T. discipes* it is clear that the relative duration of the naupliar stage is prolonged when the temperature increases" (Smol and Heip, 1974, p. 167).

Most of the above-mentioned studies deal with epiphytic or semipelagic forms, and particularly the relatively easily cultured *Tisbe* spp. Only Rao (1967) dealing with an interstitial copepod, *Arenopontia indica*, Muus (1967) with *Nitocra spinipes*, and Smol and Heip (1974) with *Paronychocamptus nanus* and *Tachidius discipes* have successfully cultured benthic harpacticoids; and there is some doubt as to the true substrate preference of *N. spinipes* and *T. discipes* (Muus, 1967). The non-sediment swelling forms can be cultured on large algal fragments such as *Ulva* or on a variety of other substrates (Purina Laboratory Rat Chow, Huizinga, 1971; yeast, Rosenfield and Coull, 1974). However, the true sediment-dwelling forms require a granular substrate for the organisms to grasp. Whether the mode of naupliar development reported here is unique to sediment-dwelling harpacticoids is open to speculation. Unfortunately, comparative studies are not found in the literature. However, since delayed naupliar development has not been reported in the many cultured semipelagic forms, one might suspect that differential larval growth is an adaptation to a benthic existence. The four species reported on here do not have pelagic nauplii, nor do they "drop" their eggs prior to hatching as reported for *Tigriopus californicus* by Huizinga (1971) and known to occur in some calanoid copepods (Zillioux and Gonzalez, 1972). Eggs mature in the egg sacs and red naupliar eyes are readily observed in the attached eggs just prior to hatching. Each nauplius hatches directly from the attached egg sac. Close observation of several specimens of *Paronychocamptus* during the hatching period revealed that the mothers actively prod and push their eggs releasing the nauplii one by one into the surrounding water. All nauplii gently settle to the substrate where some immediately start to feed and others become supine, firmly attaching themselves (ventral side down) to the substrate and are insensitive to careful probing. Most likely this dormant "settling" acts to prevent immediate growth and thus would account for the delay in naupliar development.

We do not know the causes of the delayed development nor can we test our hypothesis in the field since one needs to follow a particular individual's development. Whether the phenomenon is environmentally or genetically controlled is unknown. However, if the delayed development we have encountered in the laboratory also occurs in the field and it is genetically controlled, then those species that can differentially delay their larval development would have a great selective

advantage over those species that cannot. If a species is able to "spread out" its developmental time in response to some condition, or delay its development in response to such a condition, it allows this species to: 1) spend a greater amount of time in that particular locale, extending its ability to utilize the resources and ensure a more equitable (noncrowding) population density; and 2) essentially modify its population growth-form from that of an *r*-strategist to that of a *K*-strategist. Harpacticoid copepods have traditionally been classified as a *r*-strategists *sensu* MacArthur & Wilson (1967), Pianka (1970) and Gill (1974), *i.e.*, large families and short life cycles (Heip, 1974). Indeed, the four species described here do have large families (for harpacticoid copepods) and would be expected to be *r*-strategists. However, upon hatching and between N_1 and N_3, the ability to develop slowly and have nauplii "appear" (*i.e.*, start development a few at a time) places only a few adults in the environment at any one time and most definitely favors efficiency of conversion of food with little or no waste (*i.e.*, *K*-strategy). If the population were to be environmentally controlled (*e.g.*, food-limited) and if the nauplii all developed at once and fed on the same resource at the same time, a real possibility exists that the resource would soon become limiting. By spreading out naupliar development and therefore not over-grazing the food resource (bacteria, diatoms) on sediment particles, the food resource would essentially be kept in the log-growth phase providing sufficient food for continued (and prolonged) naupliar development.

Smol and Heip (1974) suggest that a prolongation of the naupliar stages could be selective advantage in avoiding predation. They state that nauplii are less likely captured than copepodites by the predatory polyp, *Protohydra leuckarti*. If prolongation of the naupliar stages does not increase the length of the copepodite stages, then staying in the naupliar stages longer decreases predatory effects. We do not have *Protohydra leuckarti* at our sampling sites and therefore cannot specifically suggest it as the predator. However, Proseriata turbellarians are extremely common at our sampling sites (sometimes reaching 160–200 10 cm $^{-2}$) and are known to be voracious predators on harpacticoid copepods (Reinhard M. Rieger, University of North Carolina, personal communication). If the proseriats (or perhaps some other as yet unknown predator) are functioning similarly to *Protohydra* at our sampling sites, delayed naupliar development is one way of minimizing predation on these four harpacticoid copepods.

We do not know if the differential naupliar growth is genetically or environmentally controlled. The implications of such a naupliar development are, without doubt, extremely important to the success and propagation of the species. We are continuing the work and constructing life tables of the four species, to determine the exact stage when delay takes place and also to determine more acutely the factors controlling such a delay.

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Summary

1. Four species of harpacticoid copepods maintained in culture appear to be able to inherently delay the duration of their naupliar stages.

2. Stage 1–3 nauplii continue to appear in culture up to 50 days after the mother hatches her eggs.

3. Those species with the delayed development essentially change from an *r*-strategist to a *K*-strategist during naupliar growth.

4. If delayed development also takes place in the field, then those species capable of delaying development in response to some "factor" have a distinct ecologic advantage over those that cannot.

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